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## **ALTERATIONS IN THE HEMATOLOGICAL PARAMETERS OF RAINBOW TROUT, *ONCORHYNCHUS MYKISS*, EXPOSED TO CYPERMETHRIN**

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### **Abstract**

Rainbow trout were exposed to sublethal concentrations of the pesticide cypermethrin (1/8, 1/4 and 1/2 of the LC<sub>50</sub>, 0.0082 mg/l) for 21 days at 24-hour intervals. At the end of the exposure period, hematological parameters were investigated. As the concentration of cypermethrin increased, there were increases in red blood cells, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, thrombocyte count and erythrocyte sedimentation rate. As the exposure increased, there were decreases in packed cell volume, mean corpuscular volume and white blood cell count. Differences among treatments were significant ( $p < 0.05$ ) only for red blood cells, mean corpuscular volume, thrombocyte count and erythrocyte sedimentation rate when exposure rose from 1/8 to 1/2 of the LC<sub>50</sub> concentration.

### **Introduction**

Pesticides such as deltamethrin, fenvalerate, cypermethrin and synthetic pyrethroids are widely used but adversely affect the aquatic environment to a great extent (Elliott et al., 1974; Armella et al., 1987; Santhakumar et al., 1999; Yanik et al., 2001). As a synthetic pyrethroid insecticide, cypermethrin has low toxicity to mammals but is very effective against a wide range of insects and highly toxic

to fish and other aquatic organisms (Haya, 1989; Malla Reddy and Bashamohideen, 1989; Grande et al., 1994). Little information is available on the acute toxic effects of cypermethrin in humans. In female mice, benign lung tumors occurred at the highest dose of 229 mg/kg/day, however, no tumors were observed in rats given doses up to 75 mg/kg/day (EPA, 1989).

Hematological parameters have been

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used to describe the health of fish (Blaxhall, 1972), monitor stress response (Soivio and Oikari, 1976; Kocabatmaz and Ekingen, 1984) and predict systematic relationships and physiological adaptations of animals. They more quickly reflect the poor condition of fish than other commonly measured parameters (Alkinson and Judd, 1978). Hematological parameters are known to respond quickly to changes in environmental conditions and have been studied in *Tilapia mossambica* (Aziz et al., 1993), *Ctenopharyngodon idella* (Shakoori et al., 1996) and *Heteropneustes fossilis* (Kumar et al., 1999). The objective of the present study was to determine if sub-lethal concentrations of cypermethrin change hematological parameters in rainbow trout (*Oncorhynchus mykiss*).

#### Materials and Methods

Rainbow trout ( $180 \pm 25$  g mean weight), reared in well water of a constant  $8.5^\circ\text{C}$  in our farm at the Research and Extension Center in Atatürk University, were transferred to the Central Laboratory in the Aquarium Fish Rearing Facility in April 2000. Fish were acclimated for one week in 785 l circular (100 x 100 cm) fiberglass tanks under natural light conditions with a constant flow (1.5 l/min) of aerated dechlorinated tap water at  $9-11^\circ\text{C}$  and no recirculation. The dissolved oxygen, pH and total hardness of the water were 8-9 ppm, 7.8 and 102 mg  $\text{CaCO}_3$ , respectively.

The stock solution of cypermethrin (Siperkor, Koruma Tarim Co., Turkey) was prepared in tap water, since pyrethroids are mixed with water for field application. The reported  $\text{LC}_{50}$  value for cypermethrin is 0.0082 ppm for rainbow trout (Bradbury and Coats, 1989). Sublethal doses of cypermethrin were prepared to achieve nominal tank concentrations of 0 (control), 0.001025 (1/8 of  $\text{LC}_{50}$ ), 0.00205 (1/4 of  $\text{LC}_{50}$ ) and 0.0041 (1/2 of  $\text{LC}_{50}$ ) ppm and poured into the tanks at 24-hour intervals for 21 days. The tanks were aerated by an air pump.

Three tanks, containing ten fish each, were exposed to the cypermethrin. A fourth tank containing ten fish was the control. After exposure for 21 days, three fish from the control and

eight fish from each of the treatment tanks were removed. Their blood was subjected to hematological analysis (Aziz et al., 1993; Shakoori et al., 1996; Santhakumar et al., 1999). From each fish, 2 cc venous blood was drawn using heparin as an anticoagulant. The red blood cell (RBC) and white blood cell (WBC) counts were estimated according to Blaxhall and Daisley (1973), the hemoglobin (Hb) concentration, thrombocyte count (Plt) and erythrocyte sedimentation rate according to Kocabatmaz and Ekingen (1984) and the packed cell volume (PCV) according to Schalm et al. (1975). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Malla Reddy and Bashamohideen (1989).

Results are presented as means  $\pm$  SE. Differences between parameters were analyzed by one-way analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) at  $\alpha = 0.05$  level (SAS, 1996).

#### Results

RBC, Hb, MCH, MCHC, Plt and erythrocyte sedimentation rate increased, whereas PCV, MCV and WBC decreased, with the increase in concentration of the pesticide (Table 1). There were significant differences in RBC ( $F = 5.98$ ), Hb ( $F = 28.51$ ), Plt count ( $F = 4.72$ ), MCHC ( $F = 20.68$ ), erythrocyte sedimentation rate ( $F = 7.98$ ), MCV ( $F = 19.63$ ), and PCV ( $F = 23.81$ ). There were no significant differences in levels of MCH ( $F = 1.52$ ) and WBC ( $F = 0.30$ ), between the treatments ( $p > 0.05$ ).

#### Discussion

Significant differences were observed in RBC, it increased with the increase in concentration of cypermethrin. Aziz et al. (1993) also observed an increase in erythrocyte (RBC) count in *T. mossambica* from  $1.2 \pm 0.014$  to  $1.28 \pm 0.01$  after exposure to cadmium. A similar increase (from  $1.16 \pm 0.08$  to  $1.48 \pm 0.05$ ) was observed in *C. idella* after exposure to fenvalerate (Shakoori et al., 1996) and in *H. fossilis* (from  $4.6 \pm 1.2$  to  $6.5 \pm 1.5$ ) after exposure to deltamethrin (Kumar et al., 1999). On

Table 1. Hematological parameters of rainbow trout exposed to cypermethrin for 21 days at 24-hour intervals.

	Exposure concentration ( $LC_{50} = 0.0082 \text{ ppm}$ )			
	No exposure (Control; n=3)	1/8 $LC_{50}$	1/4 $LC_{50}$	1/2 $LC_{50}$
RBC - Red blood corpuscle count ( $10^6/\text{mm}^3$ )	0.60 ± 0.10 <sup>b</sup>	0.62 ± 0.06 <sup>b</sup> (+3.33)	0.66 ± 0.06 <sup>ab</sup> (+10.0)	0.74 ± 0.06 <sup>a</sup> (+23.33)
Hb - Hemoglobin concentration (g/100 ml)	6.87 ± 0.90 <sup>b</sup>	8.80 ± 0.56 <sup>ab</sup> (+28.09)	9.20 ± 0.56 <sup>a</sup> (+33.91)	10.48 ± 0.56 <sup>a</sup> (+52.54)
PCV - Packed cell volume (%)	44.67 ± 3.51 <sup>b</sup>	43.75 ± 2.12 <sup>ab</sup> (-2.05)	39.75 ± 2.12 <sup>a</sup> (-11.01)	35.13 ± 2.12 <sup>a</sup> (-21.36)
MCV - Mean corpuscular volume ( $\text{pm}^3$ )*	751.90 ± 67.16 <sup>c</sup>	711.51 ± 90.27 <sup>c</sup> (-5.37)	603.59 ± 69.10 <sup>b</sup> (-19.72)	475.53 ± 42.70 <sup>a</sup> (-36.75)
MCH - Mean corpuscular hemoglobin (ng)*	117.60 ± 32.22 <sup>a</sup>	143.22 ± 19.67 <sup>a</sup> (+21.78)	139.54 ± 15.10 <sup>a</sup> (+18.65)	142.06 ± 15.38 <sup>a</sup> (+20.80)
MCHC - Mean corpuscular hemoglobin concentration (g/100 ml)*	15.51 ± 2.99 <sup>b</sup>	20.16 ± 1.75 <sup>ab</sup> (+29.98)	23.17 ± 1.35 <sup>a</sup> (+49.39)	29.94 ± 2.76 <sup>a</sup> (+93.03)
WBC - White blood cell count ( $10^4/\text{mm}^3$ )	6.53 ± 1.17 <sup>a</sup>	6.48 ± 0.72 <sup>a</sup> (-0.76)	6.15 ± 0.72 <sup>a</sup> (-5.82)	5.61 ± 0.72 <sup>a</sup> (-14.09)
Plt - Thrombocyte count ( $10^4/\text{mm}^3$ )	0.90 ± 0.32 <sup>b</sup>	0.93 ± 0.18 <sup>b</sup> (+3.33)	0.99 ± 0.18 <sup>b</sup> (+10)	1.63 ± 0.18 <sup>a</sup> (+81.11)
Erythrocyte sedimentation rate (mm/h)	0.33 ± 0.22 <sup>c</sup>	0.90 ± 0.13 <sup>b</sup> (+172.72)	1.00 ± 0.13 <sup>b</sup> (+203.03)	1.50 ± 0.13 <sup>a</sup> (+354.54)

Superscripts in a row with different letters represent significant differences ( $p < 0.05$ ).

Values are means ± SE of eight fish, except for the control (n=3).

Values in parentheses are differences (in %) from the control.

\*MCV = PCV/RBC

MCH = Hb/RBC

MCHC = Hb/PCV

the other hand, RBC declined in *T. mossambica* after 30 days of exposure to sumithion and sevim (Koundinya and Ramamurti, 1979), in *C. carpio* (from  $2.79 \pm 0.11$  to  $1.13 \pm 0.08$ ) after 48 hours exposure to cypermethrin (Malla Reddy and Bashamohideen, 1989) and in *Anabas testudineus* exposed to monocrotophos (Santhakumar et al., 1999). Stress caused an imbalance of electrolytes that resulted in RBC exosmosis and decreased cell size (Soivio and Oikari, 1976).

The increase in RBC count may explain the increased content of MCH. Shakoori et al. (1996) reported a decrease in the WBC count of *C. idella* exposed to fenvalerate. After insecticide intoxication, the RBC/WBC ratio increased. Perhaps, the RBC rose while the WBC remained unaffected during treatment, in accordance with the findings of Shakoori et al. (1996).

Similar to our findings, exposure to danitol and fenvalerate caused a significant reduction in the hematocrit (PCV) value in *C. idella* (Ahmad et al., 1995; Shakoori et al., 1996). However, an increase was observed in *T. mossambica* exposed to mercury chloride (Aziz et al., 1993). In spite of a significant increase in the RBC count, the significant decrease in PCV shows the magnitude of the shrinking cell size due to insecticide intoxication (Ahmad et al., 1995). The decrease in PCV and MCV show that cypermethrin may interfere with the normal physiology of RBC.

The increase in Hb concentration may be attributed to the fact that the oxygen carrying capacity of the fish was affected by the cypermethrin. Cypermethrin appears to interfere with the ability to bind hemoglobin to oxygen during respiration. Due to an insufficient supply of oxygen, respiration was not maintained efficiently. As a result, the demand for hemoglobin content increased. The increased Hb content may also be attributed to increased erythropoiesis and hemoglobin synthesis which, in turn, explains the increased MCHC. However, in freshwater catfish (*H. fossilis*), the hemoglobin (%) decreased after 30 days exposure to deltamethrin (Kumar et al., 1999).

Haniffa and Vijavarani (1989) studied the effects of textile mill effluents on hematological

parameters of freshwater *Oreochromis mossambicus* and found that an increase of the toxic concentration produced dose-dependent increases in the RBC count and hemoglobin content and decrease in the MCV. The decreased MCV and increased MCHC are indicative of hypochronic microcytic anemia. Kocabatmaz and Ekingen (1984) reported that the thrombocyte (Plt) count was  $2.1 \times 10^4/\text{mm}^3$  before stress and  $4.3 \times 10^4/\text{mm}^3$  after stress. Therefore, the increased thrombocyte in the present study can be explained by the stress-causing role of the cypermethrin.

The erythrocyte sedimentation rate increased as the dose of cypermethrin rose. Kumar et al. (1999) found that the erythrocyte sedimentation rate increased from  $6.0 \pm 2$  mm/h to  $8.5 \pm 1.8$  mm/h in *H. fossilis* exposed to deltamethrin. The increase in erythrocyte sedimentation rate shows that the fish were heavily intoxicated by the increased concentration of cypermethrin.

The physiological and chemical properties of fish blood are very sensitive to environmental changes (Hughes and Nemcsok, 1988; Yanik and Atamanalp, 2001). The present study reveals that cypermethrin has profound effects on the hematological parameters of rainbow trout. Further works are needed since fish at different growing stages may respond at different levels to environmental factors.

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